A Multispectral Fluorescence and Reflectance Probe for In Situ Characterization of Benthic Environments

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LONG-TERM GOAL

This work is undertaken as part of a long-term effort to understand the optical signatures of marine organisms and substrates, and to use the information to devise technology for improved mapping and assessment of seafloor features.

OBJECTIVES

The objective of this effort was to investigate the feasibility of developing a compact, low-power, low-cost probe that would remotely interrogate the fluorescence and reflectance properties of the sea floor and benthic marine organisms at a number of wavelengths. The resultant data would comprise the input to an automated bottom type classification algorithm. The point-by-point bottom classifications would in turn comprise the input to a statistical approach to characterization of the environment being surveyed. The system would be useable by divers or as a component on an autonomous or remotely operated underwater vehicle.

APPROACH

The work plan consisted of three main tasks: 1) construct and bench test a prototype of the sensor system (light sources, detectors, and data handling); 2) model the factors that would impact the operation of the sensor in the optically variable seawater environment; and 3) evaluate alternative schemes for processing and utilizing the sensor data stream. Charles Mazel of Physical Sciences Inc. (PSI) led the effort, with assistance from PSI personnel Tom Vaneck in data analysis and Mike Hinds in LabView programming.

WORK COMPLETED

We constructed a benchtop prototype consisting of two electronic flash units and four photomultiplier tube (PMT) detectors. Each unit was configured with a different interference filter, permitting fluorescence excitation at two wavelengths and detection at four. The flashes could also be operated without a filter, providing a broadband source for reflectance measurements. The triggering of the flashes and the digitization of the PMT responses were controlled by a custom LabView program. The light source outputs and the detector sensitivities were calibrated against detectors and sources with known characteristics. We then used a variety of targets with known fluorescence and reflectance properties to verify the performance of the hardware, data acquisition software, and data post-processing routines. A schematic representation of the benchtop system is shown in Figure 1.

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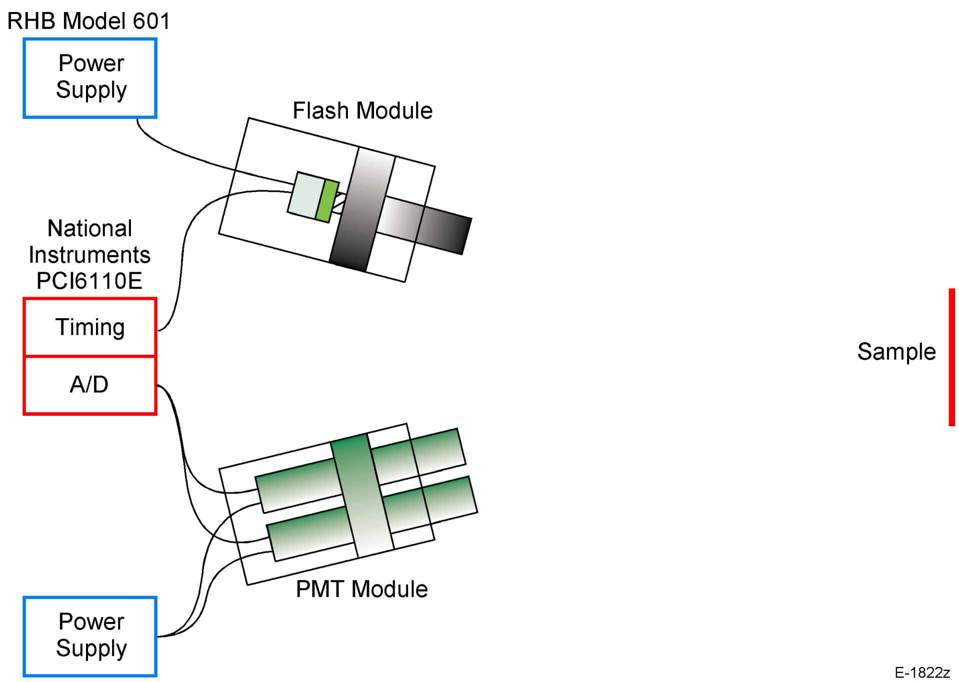

Figure 1. Top view schematic of the benchtop experimental system.

Once the system operation and data throughput were fully characterized we used the apparatus to make measurements of benthic macroalgae following the approach outlined in Topinka et al., 1990. Their paper suggested that it would be possible to distinguish between the three main classes of macroalgae – brown (Phaeophyceae), red (Rhodophyceae) and green (Chlorophyceae) – by using two different excitation wavelengths to stimulate 685 nm chlorophyll fluorescence and comparing the magnitudes of the responses. Specimens from each group were collected from subtidal locations in New England and measured while fresh. We used 460 and 540 nm interference filters on the flash units, corresponding to the excitation wavelengths suggested by Topinka et al.

We modeled the impact of water attenuation characteristics on the expected spectral signatures as a function of stand-off distance. Previously measured fluorescence and reflectance spectra were used as inputs to the model, and water inherent optical properties were taken from field data. We also considered the impact that operating factors such as probe repetition rate and vehicle speed of advance would have on the data sampling.

We experimented with several schemes for data processing, with primary focus on a fuzzy logic classifier trained using a genetic algorithm.

RESULTS

The results demonstrated the feasibility of the hardware design. The components functioned as planned and we were able to acquire fluorescence and reflectance data from a variety of test subjects in the laboratory. The system proved to be capable of accurately measuring fluorescence and reflectance spectra. Figure 2 shows a comparison of the processed data acquired by the prototype benthic probe with the fluorescence emission spectrum measured with a laboratory instrument (FluoroMax-2, SPEX

Industries). The results for reflectance measurements with the probe showed similar agreement with data from a commercial spectroradiometer.

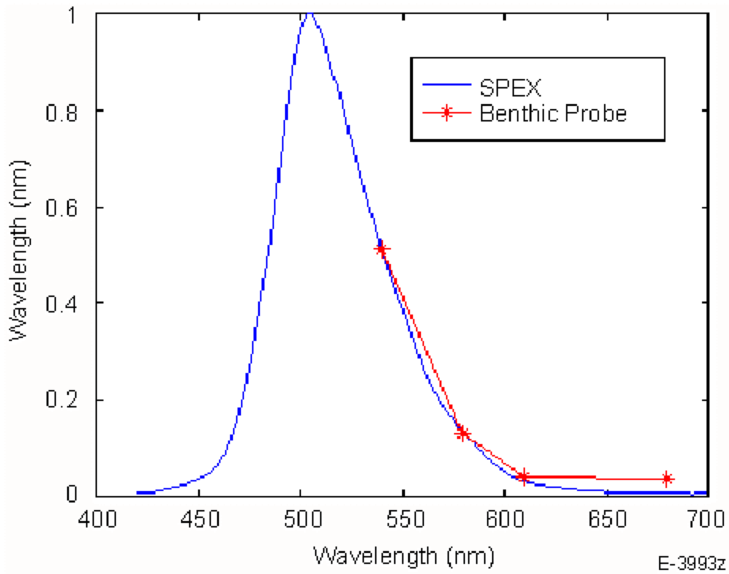

Figure 2. Comparison of fluorescence emission as measured by a laboratory spectrofluorometer (solid line) and the multispectral benthic probe.

Table 1 summarizes the results of the experiment to distinguish macroalgal groups based on the differences in their excitation spectra. The response ratios computed from the benthic probe data are remarkably close to those derived by Topinka et al. This demonstrates the potential for an in situ optical probe of this sort to distinguish among these major algal groups.

Table 1. Summary of data from multi-excitation experiment with macroalgae

Algal Group	Mean 540:465, Topinka et al.	540:460, BOP
Phaeophyceae	0.59	0.52
Chlorophyceae	0.28	0.22
Rhodophyceae	3.67	3.96

Figure 3 is a representative example of the result of modeling the effect of water path attenuation on fluorescence spectral signatures as a function of sensor-to-sample distance. Attenuation measured in a coral reef environment was used for the water properties, and the fluorescence spectrum is that of a commonly occuring fluorescent pigment in corals (Mazel, 1997). The results have been normalized to emphasize the change in spectral shape as opposed to the change in signal intensity. The emission spectrum overlaps the region where attenuation increases markedly at approximately 600 nm, with the result that the spectral shape is strongly affected by operating distance. In contrast, the fluorescence from coral pigments that fluoresce at shorter wavelengths decreases in intensity but maintains its spectral shape. This kind of result has important implications for selection of detection filter wavelengths and data processing schemes.

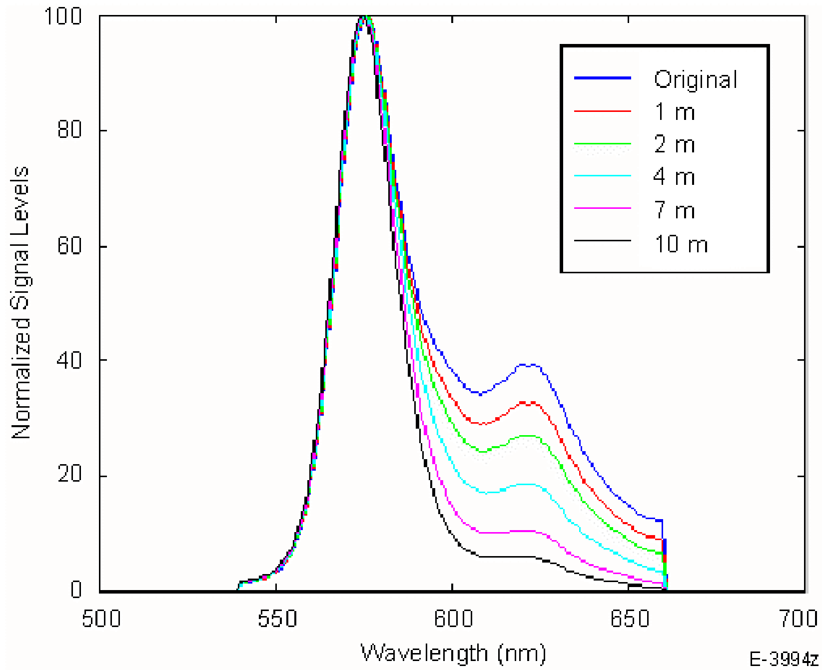

Figure 3. Effect of increasing standoff distance on the spectral shapes of the fluorescence emission for the orange-fluorescent coral pigment.

The experimentation with data classification schemes demonstrated that the multispectral data could be used in a fuzzy logic scheme to separate bottom types based on spectral characteristics. This work was done with existing three-channel fluorescence data collected by the Fluorescence Imaging Laser Line Scan system built by Raytheon Electronic Systems and operated by the Coastal Systems Station, Panama City.

IMPACT/APPLICATION

The optical probe is designed to take advantage of newly emerging knowledge of the fluorescence and reflectance spectral characteristics of various targets to develop a compact seafloor classification system suitable for use on a small remotely operated or autonomous underwater vehicle. If it were developed further it would ideally provide the capability to autonomously classify seafloor features. This could permit more consistent and frequent mapping than is currently practical. The resulting data would be of interest for resource discovery and mapping, ecological assessment, and planning of seafloor-based operations in any water depth.

TRANSITIONS

This was a Phase I benchtop effort. There are as yet no transitions to other projects.

RELATED PROJECTS

We have been investigating the fluorescence and reflectance characteristics of seafloor substrates and organisms as part of the ONR Coastal Benthic Optical Properties program. That work is adding to the database of optical properties of the sea floor that is needed to guide the choice of operating excitation and emission wavelengths for an in situ active optical probe of the type described here.

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